

# Geographic Variation of Mercury in Breeding Tidal Marsh Sparrows of the Northeastern United States

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## Research Article

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## Geographic variation of mercury in breeding tidal marsh sparrows of the northeastern United States

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### Abstract

Saltmarsh sparrows (*Ammodramus caudacuta*) and seaside sparrows (*A. maritima*) are species of conservation concern primarily due to global sea-level rise and habitat degradation. Environmental mercury (Hg) contamination may present additional threats to their reproductive success and survival. To assess site-specific total mercury (THg) exposure and identify environmental correlates of THg detection across a large portion of the breeding range where these species co-occur, we sampled blood from adult male saltmarsh and seaside sparrows at 27 marsh sites from Maine to Virginia, USA. The mean THg concentration ( $\pm 1$  SD) throughout the entire sampling range was  $0.531 \pm 0.287$   $\mu\text{g/g}$  wet weight (ww) for saltmarsh sparrows and  $0.442 \pm 0.316$   $\mu\text{g/g}$  ww for seaside sparrows. Individual THg concentrations ranged from 0.135–1.420  $\mu\text{g/g}$  ww for saltmarsh sparrows and 0.153–1.530  $\mu\text{g/g}$  ww for seaside sparrows. Model averaging from a suite of linear mixed models supported species-based differences in blood THg. On average, saltmarsh sparrows had 20.1% higher blood THg concentrations than seaside sparrows. We hypothesize that species-specific THg concentrations are influenced by differences in diet or foraging preferences between these species. We did not detect any correlations between sparrow THg concentrations and land cover characteristics surrounding sampled marshes or cumulative average annual precipitation.

**Key Words:** *Ammospiza caudacuta*, *Ammospiza maritima maritima*, bioindicator, estuary, mercury, salt marsh

### **Declarations**

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*Conflicts of interest:* The authors declare that they have no conflict of interest.

*Ethics approval (include appropriate approvals or waivers):* Sampling was conducted under approved animal care protocols from the State University of New York: College of Environmental Science and Forestry (180201), University of Connecticut (A17-011), and University of Maine (A2018-03-03).

*Consent to participate (include appropriate statements):* Not applicable.

*Consent for publication (include appropriate statements):* Not applicable.

*Availability of data and material/ code availability:* All data and software are archived and available at <https://github.com/csayers2/Sayers-Ammospiza-Hg-2021> or <https://www.mrlc.gov/data>.

*Authors' contributions:* Christopher J. Sayers II and David N. Bonter contributed to the study conception and design. Material preparation and data collection were performed by Christopher J. Sayers II, Mackenzie R. Roeder, Lindsay Forrette, Daniel Roche, Sam E. Apgar, Alison R. Kocek, Alexandra M. Cook, W. Gregory Shriver, Chris S. Elphick, Brian Olsen, and David N. Bonter. Christopher J. Sayers II, Gaetan L. B. Dupont, David N. Bonter, and Chris S. Elphick conducted and contributed to analyses. The first draft of the manuscript was written by Christopher J. Sayers II and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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## Introduction

Mercury (Hg) is a widespread environmental contaminant that can pose a threat to wildlife by affecting neurological and reproductive systems (Evers 2018). Common anthropogenic sources of inorganic Hg include coal-fired power plants, waste incinerators, chlorine and metal processing plants, landfills, and artisanal and small-scale gold mines that emit Hg into the atmosphere or directly into waterways (UNEP 2019). Once deposited into aquatic systems, either by atmospheric processes or leachate, inorganic Hg can be converted into the neurotoxic and bioavailable methylmercury (MeHg). Generally, organisms that occupy higher trophic levels are disproportionately affected by MeHg because it is lipophilic and migrates through food webs via biomagnification (Evers et al. 2005). Increased concentrations of MeHg in tissue samples of piscivorous and insectivorous birds are associated with impaired coordination, foraging apathy, reduced reproductive success, and reduced survivorship (Scheuhammer 1987; Bouton et al. 1999; Evers et al. 2008).

In anoxic aquatic sediments, sulfate-reducing bacteria accelerate the production of MeHg (Compeau and Bartha 1985), leading to elevated risks for organisms living in, around, or feeding from contaminated wetlands. Both the saltmarsh sparrow (*Ammospiza caudacuta*) and seaside sparrow (*A. maritima*) are tidal marsh specialists and spend their entire life cycles within saltmarsh habitats, where risk of exposure to MeHg can be elevated. Being dependent on salt marshes, which are under increasing threat of degradation and loss, tidal marsh sparrows are species of high conservation concern (Rosenberg et al. 2016). Anthropogenic activities including coastal development, marsh fragmentation, and alteration of hydrology have all reduced habitat available for both saltmarsh and seaside sparrows (Greenlaw et al. 2020; Post and Greenlaw

2020), and global sea-level rise threatens saltmarsh systems range-wide (Bayard and Elphick 2011; Field et al. 2017; Greenlaw et al. 2020; Post and Greenlaw 2020). The saltmarsh sparrow is considered “globally endangered” by the International Union for Conservation of Nature in response to rapid population declines (Birdlife International 2018), decreasing at 9% per year with over 70% of the global population lost since the 1990s (Correll et al. 2017; Roberts et al. 2019). Sea-level rise and increased tidal flooding risk are the major drivers of population loss for this species, as approximately 60% of all nest failures occur due to flooding events (Shriver et al. 2007; Gjerdrum et al. 2008), and are projected to cause extinction as early as 2035 (Field et al. 2017, 2018; Roberts et al. 2019). Because salt marshes and coastal estuaries are often impacted by polluted urbanized watershed runoff (Schwarzbach et al. 2006), heavy metal contamination may present additional challenges to sparrow reproductive success and survival.

Tidal marsh sparrows can be effective bioindicators for MeHg contamination of saltmarsh systems, and for quantifying the potential threat that MeHg poses to marsh bird populations, at a variety of geographic scales (Shriver et al. 2006; Warner et al. 2010; Lane et al. 2011, 2020; Winder 2012; Ruskin et al. In prep.). In all prior research, tidal marsh sparrow blood Hg levels have approached or exceeded 0.7  $\mu\text{g/g}$  wet weight (Shriver et al. 2006; Lane et al. 2011, 2020; Warner et al. 2010; Winder 2012; Ruskin et al. In prep.), a level that has been shown to influence nesting success in Carolina Wren (*Thryothorus ludovicianus*) (Jackson et al. 2011). Though atmospheric deposition of Hg via precipitation is known to account for a sizeable portion of the Hg present in the environment (Hudson et al. 1995), breeding tidal marsh sparrow Hg concentrations in the northeastern US vary significantly by site (Warner et al. 2010; Lane et al. 2011, 2020; Ruskin et al. In prep.) without any consistent temporal trends (Lane et al. 2020).

These findings suggest that regional atmospheric deposition alone does not drive Hg bioavailability in coastal wetlands of the northeastern USA. Rather, variation in tidal marsh sparrow Hg levels among marsh sites is more likely due to site-specific MeHg production rates, differences in marsh hydrodynamics or biogeochemistry, differences in local food webs and food availability, or nearby point sources of the pollutant and surrounding land use (Lane et al. 2011, 2020).

Little is known about the dynamics of Hg in tidal wetlands or how local land cover influences Hg levels. Wetland-associated dissolved organic carbon (DOC) is positively correlated with the presence, production, and export of MeHg in the hydrological system (St. Louis et al. 1994; Krabbenhoft et al. 1995; Driscoll et al. 1995, 1998; Wiener and Shields 2000). Trees absorb atmospheric Hg through leaf stomata and may deposit Hg into terrestrial and aquatic systems during litterfall, or enhance the Hg concentration of precipitation as it falls through the canopy (Rea et al. 1996). Sources of direct Hg discharges, such as metal-processing plants, landfills, and waste-water treatment plants, tend to aggregate in urbanized environments and are linked to Hg contamination within drainage basins (Niebla et al. 1976; Hildebrand et al. 1980; Gilmour and Bloom 1995). Impervious surfaces also direct Hg to surface water via storm water drainage systems (Rule et al. 2006). Therefore, we predicted that MeHg levels in marshes and marsh-associated birds would be affected by the relative amount of wetlands, forests, and developed areas in the surrounding landscape.

Range-wide analyses of Hg contamination are lacking for most species, including saltmarsh and seaside sparrows. Within their co-occurring breeding ranges, three major geographic gaps in Hg sampling exist: coastal New Jersey, coastal Delmarva, and the eastern

Chesapeake Bay. These subregions collectively support over 65% of both the global saltmarsh sparrow population and the northeastern seaside sparrow (*A. m. maritima*) population (Wiest et al. 2016). Consequently, the objectives of our study were to, 1) quantify regional variation in the mercury detected in saltmarsh and seaside sparrows across a large portion of their breeding ranges in the northeastern USA, and 2) identify environmental and spatial characteristics that influence patterns of mercury detection. We sought to conduct an assessment of local mercury contamination, which could potentially inform conservation and remediation efforts.

## **Methods**

### *Sample collection*

To quantify regional variation of MeHg exposure in saltmarsh and seaside sparrows, we collected blood samples from 67 adult male saltmarsh sparrows and 53 adult male seaside sparrows at 27 marsh sites along the coast of the northeastern and mid-Atlantic USA from June 1 to July 31, 2018. Marsh sites were located from Maine south to the Delmarva Peninsula of Virginia (Fig. 1, Table 1) and were selected using a randomization process that first used generalized random tessellation stratified sampling to generate bird survey points (Wiest et al. 2016). Points were then randomly subsampled after stratifying by sparrow abundance (see “Rapid Demo SOP,” [www.tidalmarshbirds.org](http://www.tidalmarshbirds.org)). Because blood samples are indicative of days to weeks of dietary exposure (Evers 2018) and reliably represent site-specific MeHg availability, they are an appropriate sampling tissue to understand tidal marsh sparrow exposure to MeHg in the local environment (Evers et al. 2005; Shriver et al. 2006). Tidal marsh sparrows begin their prebasic molt in mid- to late August (Greenlaw et al. 2020; Post and Greenlaw 2020); therefore,

sampling during June and July removes the confounding possibility of birds shunting their MeHg load into feathers during molt. We only sampled adult males to avoid the potential confounding factors associated with females passing on their Hg body burden to eggs during the laying period (Brasso et al. 2010; Ackerman et al. 2013; Ou et al. 2015).

Sparrows were captured within a 50 m radius of a given site using mist nets and fitted with an aluminum US Fish and Wildlife Service leg band. Individuals identified as after-hatch-year males via a cloacal protuberance were weighed using a digital scale, and then 10–50  $\mu\text{L}$  of blood were collected in 70  $\mu\text{L}$  heparinized capillary tubes using brachial venipuncture. Capillary tubes were immediately sealed at both ends using Leica Microsystems Critocaps™, placed into a Vacuette™, and stored in a cooler with ice packs. Samples were transferred into a freezer within 8 hours and were maintained below  $-4\text{ }^{\circ}\text{C}$  until analysis.

### *Laboratory analysis*

Blood samples were analyzed for total Hg (THg), the combination of methylated and unmethylated Hg in the blood, at the Texas A&M University Trace Element Research Laboratory in College Station, Texas using a combustion, gold trapping, atomic absorption spectroscopy approach. This analysis was performed using a Nippon MA-3000 direct thermal decomposition Hg analyzer equipped with dual cell detectors. THg was analyzed instead of MeHg because Rimmer et al. (2005) showed that the total Hg in songbird blood is composed of approximately 95% MeHg. Samples were thawed, removed from capillary tubes, weighed to the nearest 0.01 mg, transferred to pre-combusted cuvettes, and then placed into the Nippon MA-3000's autosampler. Samples were dried and combusted under a constant flow of  $\text{O}_2$ ,

combustion gases were passed through a gold trap to remove Hg gas from the combustion stream, and the gold trap was then heated to release concentrated Hg<sup>0</sup> to be swept into two atomic absorption cells. THg concentrations in samples were quantified by comparing absorption peaks with known calibration standards. Instrument calibration was performed using a blank and three calibration standards in both detector cells and verified every 10 samples using two certified reference materials and a continuing calibration blank. Reference materials NIST 2976 and ERM-CE464 were used to evaluate the calibration on the more sensitive and less sensitive cells, respectively. This approach has been incorporated by the United States Environmental Protection Agency (EPA) in EPA SW-846 Method 7473 for analyzing solid waste and leachate (USEPA 1998). Instrument detection limit was 0.05 ng THg and final results for THg concentration are reported in µg/g wet weight (ww).

### *Statistical approach*

Using an information-theoretic approach, we built two linear mixed model candidate sets to determine a statistically relevant spatial extent for land cover extraction, as well as which spatial and environmental parameters are associated with blood THg variation in tidal marsh sparrows among marsh sites. We selected independent variables based on a set of *a priori* hypotheses (Burnham and Anderson 2002) derived from known dynamics of Hg in the environment, and scaled these variables ( $\mu = 0$ ,  $sd = 1$ ) because raw values occurred at different orders of magnitude. We constructed models in R (v. 3.6.2; R Core Team 2019) using the package “lmerTest” (Kuznetsova et al. 2017), and fit models with maximum likelihood

estimations (Zuur et al. 2009). Model selection was performed using second-order Akaike's Information Criterion for small-sample sizes ( $AIC_C$ ; Burnham and Anderson 2002).

We inspected the distribution of the blood THg concentrations for normality using quantile-quantile plots and a Shapiro-Wilk normality test, and rejected the null hypothesis that the data were normally distributed ( $W = 0.881$ ,  $p < 0.001$ ). To achieve normality, we used a natural log-transformation ( $W = 0.979$ ,  $p = 0.054$ ). Four high outliers were present prior to transformation, but were included in the analysis because we had no reason to believe they were erroneous. Following transformation, there was no evidence that homogeneity of variance, normality of residuals, and normality of random effects assumptions were violated, based on visually inspecting quantile-quantile plots and plotted residuals versus predicted values (Zuur et al. 2009).

To populate the full linear model, we extracted 30 x 30 m resolution land cover data (percent of total area) around each sampling point from the National Land Cover Database 2016 CONUS data (NLCD, Yang et al. 2018) and lumped land cover types into 3 relevant categories: wetland (NLCD categories 90 and 95 combined), forest (NLCD categories 41, 42, 43, and 52 combined), and developed land (NLCD categories 21, 22, 23, and 24 combined). We also included cumulative average annual precipitation data around each sampling point at a 30-second resolution sourced from WorldClim Version 2.0 Bioclimatic variable "BIO12" using the R package "raster" (Hijmans 2018) to account for potential differences in deposition rates throughout the sampling range. We included species as a fixed effect because the uptake of MeHg occurs mainly through diet; and the interspecific foraging differences and relative size differences between species (Woolfenden 1968) could influence MeHg uptake as measured in

the blood. To account for geographic variation in Hg deposition, prey availability, marsh hydrology, and other spatially variable conditions, we initially included latitude and longitude as fixed effects, and included marsh site as a random intercept in all models. When investigating appropriate spatial scales for the analysis (see below), we discovered that some of our variables were highly correlated at larger spatial scales, as indicated by variation inflation factors ( $VIF > 3$ ) and correlation coefficients ( $R^2 > 0.7$ ) using R package “usdm” (Hair et al. 2010; Naimi et al. 2014). After removing latitude, longitude, and percent area of wetlands from the full model, there was no evidence of multicollinearity at any of the spatial scales we evaluated.

To test for correlations between tidal marsh sparrow THg concentrations and landscape characteristics surrounding sampling points, we attempted to define a statistically relevant spatial extent, or “scale of effect,” for the independent variables. To accomplish this, we compared linear mixed models of identical structure, but with spatial attributes extracted within different buffer radii (Jackson and Fahrig 2012). Wiener and Shields (2000) demonstrated that industrially contaminated wetlands produced and exported MeHg 25 km downstream from the Sudbury River, Massachusetts, and Hildebrand et al. (1980) detected noticeable levels of THg in crayfish 130 km downstream from a chemical processing plant. Thus, we selected and compared buffer radii at 1-km increments from 1–10 km and at 5-km increments from 15–50 km to explore a range of potentially relevant scales. The model used to compare scales contained independent variables that would reasonably be influenced by radius selection: developed and forested land cover, in addition to cumulative average annual precipitation, within a 1–50 km radius around each sampling point. We defined the scale of effect to be the buffer radius that resulted in the lowest model  $AIC_C$  value.

To investigate which spatial and environmental parameters affect blood THg variation in tidal marsh sparrows, we constructed a set of 16 candidate models, which included a global model with all fixed effects, all possible reduced model combinations, as well as a null model with only site as a random effect. Fixed effects represented *a priori* hypotheses that tidal marsh sparrow blood THg concentrations are affected by the percentage of forested or developed land surrounding each sampling point, the species, and the cumulative average annual precipitation surrounding each point via its effect on atmospheric deposition. We averaged all models in the candidate set to obtain coefficient estimates for each independent variable using “MuMIn” (Bartón 2019). We then estimated 95% confidence intervals and summed Akaike weights ( $w_i$ ) across all models containing each fixed effect to determine whether each independent variable affected THg levels. If a parameter had a 95% CI that overlapped zero and a summed  $w_i < 0.75$ , we concluded that our data provided little support for a relationship between the variable and blood THg in marsh sparrows (Smith et al. 2018).

## Results

Throughout the sampling range, the mean blood THg concentration ( $\pm 1$  SD) was  $0.531 \pm 0.287$   $\mu\text{g/g ww}$  for saltmarsh sparrows ( $n = 67$ ) and  $0.442 \pm 0.316$   $\mu\text{g/g ww}$  for seaside sparrows ( $n = 53$ ). Individual THg concentrations ranged from 0.135–1.420  $\mu\text{g/g ww}$  for saltmarsh sparrows and 0.153–1.530  $\mu\text{g/g ww}$  for seaside sparrows. We detected the lowest mean sparrow concentrations at Idlewild Park, New York ( $0.187 \pm 0.068$   $\mu\text{g/g ww}$ ) and the highest at Edwin B. Forsythe National Wildlife Refuge - Great Bay, New Jersey ( $1.057 \pm 0.341$   $\mu\text{g/g ww}$ ) (Table 1, Fig. 1).

Models with buffer radii of 20–45 km were statistically indistinguishable ( $\Delta AIC_C < 2$ , Appendix 1), however, a 30-km buffer resulted in the lowest  $AIC_C$  value, and was thus chosen as the most appropriate scale for describing the landscape. The top five models in our candidate set, accounting for 62% of total model weight, were statistically indistinguishable ( $\Delta AIC_C < 2$ , Table 2). Species was included as a fixed effect in all six top-performing models, and the model containing species and a random site effect had 5.4 times more weight than the model with the random site effect alone, suggesting interspecific differences in blood THg concentrations. Adding other independent variables did not result in better supported models (Table 2); which is consistent with the finding that, after conditional model averaging, all independent variables except for species had estimated 95% confidence intervals that included zero (Table 3).

**Table 1** Location information, sample sizes (n), and species blood total mercury (THg) concentrations (mean  $\pm$  SD and range) for all sampling locations, Maine to Virginia, USA, June 1 to July 31, 2018. A dash (–) indicates that there are no data to report

Location (latitude, longitude)	Saltmarsh sparrow THg ( $\mu\text{g/g ww}$ )			Seaside sparrow THg ( $\mu\text{g/g ww}$ )		
	n	Mean $\pm$ SD	Range	n	Mean $\pm$ SD	Range
South Thomaston, Maine (44.077999, –69.142495)	6	0.51 $\pm$ 0.14	0.37–0.78	—	—	—
Scarborough Marsh WMA, Maine (43.565488, –70.365070)	3	0.33 $\pm$ 0.07	0.27–0.40	—	—	—
Rachel Carson NWR - Eldridge Rd., Maine (43.292158, –70.577234)	4	0.46 $\pm$ 0.09	0.34–0.53	—	—	—
Rachel Carson NWR - Furbish Rd., Maine (43.280176, –70.584196)	4	0.45 $\pm$ 0.18	0.31–0.71	—	—	—
Barn Island WMA, Connecticut (41.337533, –71.866209)	4	0.64 $\pm$ 0.03	0.61–0.67	1	0.52	—
Quonochontaug, Rhode Island (41.333557, –71.716765)	4	0.36 $\pm$ 0.05	0.31–0.42	—	—	—
Idlewild Park, New York (40.653427, –73.752154)	1	0.13	—	2	0.21 $\pm$ 0.07	0.16–0.26

Gilgo State Park, New York (40.642785, -73.319184)	4	0.72 ± 0.10	0.62–0.85	1	0.75	—
Marine Nature Study Area, New York (40.621389, -73.621598)	3	0.79 ± 0.18	0.60–0.96	—	—	—
North Cinder Island, New York (40.611991, -73.610016)	1	1.06	—	2	0.92 ± 0.14	0.83–1.02
North Cinder Island, New York (40.605962, -73.611136)	1	0.95	—	2	0.64 ± 0.05	0.60–0.68
Conaskonk Point, New Jersey (40.455750, -74.176680)	3	0.34 ± 0.02	0.33–0.36	2	0.33 ± 0.07	0.28–0.38
Wrangle Creek, New Jersey (39.837090, -74.150070)	3	0.85 ± 0.12	0.71–0.95	3	1.10 ± 0.35	0.78–1.48
Edwin B. Forsythe NWR - Manahawkin, New Jersey (39.691680, -74.205150)	3	0.66 ± 0.30	0.48–1.01	1	0.53	—
Edwin B. Forsythe NWR - Great Bay, New Jersey (39.527792, -74.444502)	3	1.09 ± 0.25	0.88–1.36	3	1.02 ± 0.48	0.58–1.53
Cohansey River WMA, New Jersey (39.366050, -75.358540)	—	—	—	3	0.57 ± 0.08	0.49–0.65
New Sweden WMA, New Jersey (39.310620, -75.233730)	—	—	—	3	0.23 ± 0.04	0.20–0.27
Slaughter Beach, Delaware (38.895236, -75.290328)	3	0.94 ± 0.42	0.67–1.42	3	0.57 ± 0.04	0.53–0.60
Prime Hook NWR, Delaware (38.815090, -75.229770)	3	0.49 ± 0.07	0.44–0.57	3	0.34 ± 0.05	0.29–0.40
Rehoboth Bay, Delaware (38.693920, -75.138890)	—	—	—	3	0.23 ± 0.08	0.17–0.31
Fishing Bay WMA - Elliot Island Rd., Maryland (38.351900, -75.936660)	3	0.24 ± 0.05	0.19–0.28	3	0.26 ± 0.02	0.24–0.28
Fishing Bay WMA - Great Marsh Point, Maryland (38.350410, -75.961750)	3	0.21 ± 0.02	0.19–0.24	2	0.17 ± 0.03	0.15–0.20
Fishing Bay WMA - Cedar Creek Rd., Maryland (38.318790, -76.052950)	2	0.27 ± 0.08	0.21–0.33	3	0.29 ± 0.05	0.25–0.35
Deal Island WMA, Maryland (38.162700, -75.866040)	—	—	—	3	0.32 ± 0.12	0.19–0.43
Johnson Bay - Taylor Landing, Maryland (38.078150, -75.363830)	3	0.22 ± 0.01	0.21–0.23	3	0.19 ± 0.05	0.16–0.25

Johnson Bay - Sandy Point, Maryland (38.069520, -75.369890)	2	0.23 ± 0.03	0.21–0.25	3	0.17 ± 0.01	0.15–0.18
Chincoteague NWR, Virginia (37.973850, -75.327360)	1	0.38	—	3	0.33 ± 0.09	0.22–0.42

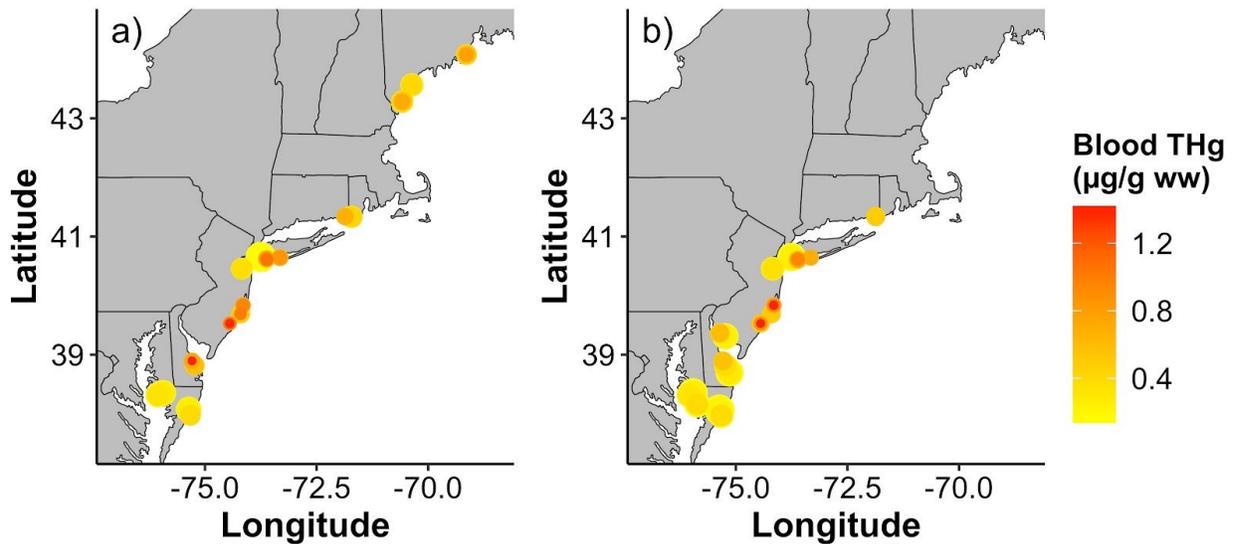
**Table 2** Model-selection results examining the effects of spatial, environmental, and biological parameters on the natural log-transformed blood THg concentrations ( $\mu\text{g/g ww}$ ) of breeding tidal marsh sparrows in salt marshes from Maine to Virginia, USA, June 1 to July 31, 2018. Site is a random variable in all models as indicated by (1 | Site)

Model	AIC <sub>C</sub>	$\Delta\text{AIC}_C$	$w_i$	−logL	K
Developed + Forest + Precipitation + Species + (1   Site)	83.594	0.000	0.224	−34.297	7
Developed + Forest + Species + (1   Site)	85.042	1.447	0.109	−36.149	6
Developed + Species + (1   Site)	85.071	1.477	0.107	−37.273	5
Species + (1   Site)	85.387	1.792	0.092	−38.519	4
Developed + Precipitation + Species + (1   Site)	85.563	1.968	0.084	−36.409	6
Precipitation + Species + (1   Site)	85.619	2.0245	0.081	−37.547	5
Developed + Forest + Precipitation + (1   Site)	85.666	2.072	0.079	−36.462	6
Forest + Precipitation + Species + (1   Site)	86.237	2.642	0.059	−36.747	6
Forest + Species + (1   Site)	87.009	3.415	0.041	−38.242	5
Developed + Forest + (1   Site)	87.453	3.859	0.033	−38.464	5
Developed + (1   Site)	88.432	4.837	0.019	−40.042	4
(1   Site)	88.804	5.209	0.017	−41.298	3
Forest + Precipitation + (1   Site)	88.980	5.386	0.015	−39.227	5
Developed + Precipitation + (1   Site)	88.985	5.389	0.015	−39.229	5
Precipitation + (1   Site)	89.101	5.506	0.014	−40.376	4
Forest + (1   Site)	89.976	6.381	0.009	−40.814	4

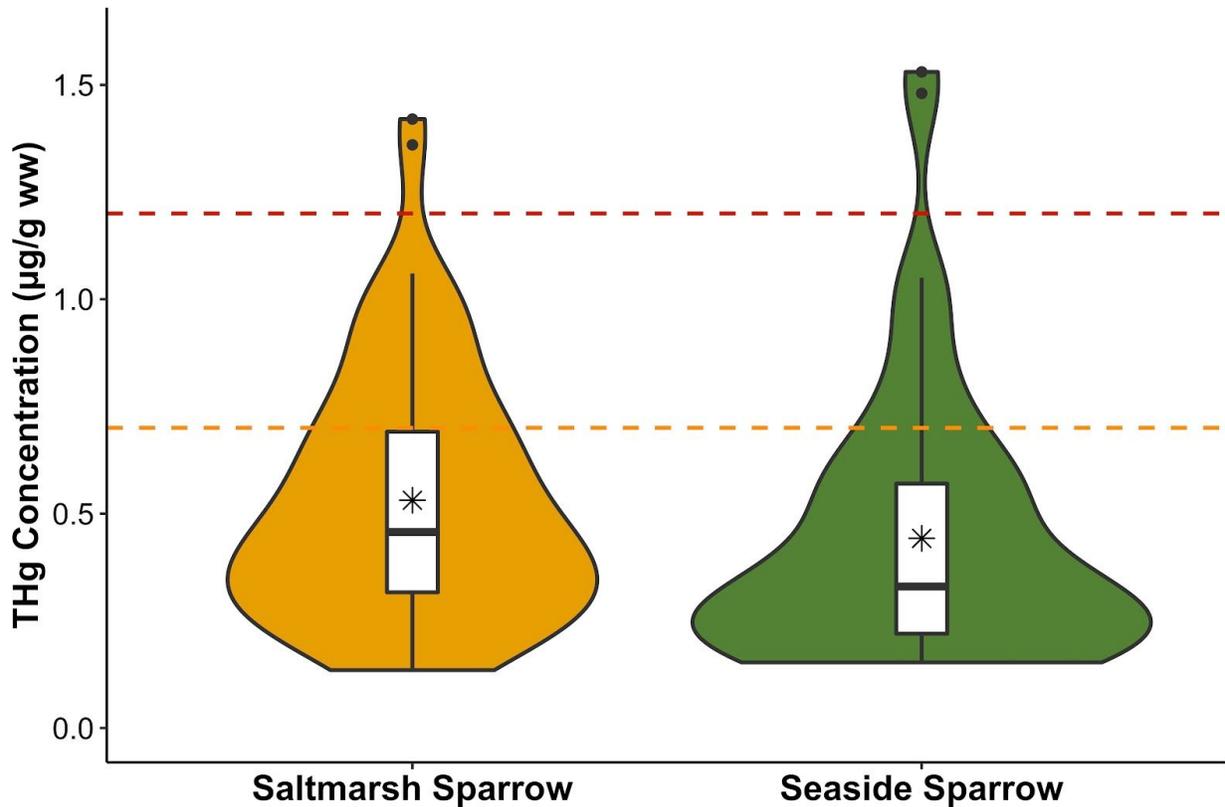
**Table 3** Conditional model-averaged parameter estimates ( $\pm$  adjusted SE), unconditional 95% confidence intervals, summed Akaike weights ( $w_i$ ), p-values, VIF scores, as well as unscaled means ( $\pm 1$  SD) and ranges for each parameter included in the candidate set. There was no evidence of multicollinearity, as indicated by VIF values  $< 3$  from the full model

Parameter	VIF	Mean ( $\pm 1$ SD)	Range	Estimate $\pm$ SE	95% CI	$w_i$	$p$
% developed land	1.099	0.134 $\pm$ 0.139	0.024–0.479	0.186 $\pm$ 0.09	–0.003 to 0.374	0.67	0.054
% forest	1.159	0.139 $\pm$ 0.096	0.031–0.319	0.177 $\pm$ 0.108	–0.035 to 0.389	0.57	0.101
Precipitation (mm)	1.057	2772994 $\pm$ 456396	1820447–3751937	–0.163 $\pm$ 0.097	–0.353 to 0.026	0.57	0.092
Species*	—	—	—	–0.121 $\pm$ 0.054	–0.227 to –0.015	0.80	0.026

\* Modeling estimate for seaside sparrow in reference to saltmarsh sparrow



**Fig. 1** Map of blood THg concentrations ( $\mu\text{g/g ww}$ ) of breeding a) saltmarsh sparrows and b) seaside sparrows for all sampling locations from Maine to Virginia, USA, June 1 to July 31, 2018



**Fig. 2** Blood THg concentrations ( $\mu\text{g/g ww}$ ) of breeding tidal marsh sparrows from Maine to Virginia, USA, June 1 to July 31, 2018. The “violin” shapes represent the vertically-oriented distribution of THg concentrations per species. The boxplots display the outliers (dots), maximum and minimum concentration excluding the outliers (ends of whiskers), first and third quartile (central box), median (horizontal black line), and mean (\*) THg concentration per species. Dashed horizontal lines indicate the  $0.7 \mu\text{g/g ww}$  10% effect concentration (EC10) and the  $1.2 \mu\text{g/g ww}$  20% effect concentration (EC20) defined by Jackson et al. (2011)

## Discussion

Our conditional model-averaged results indicated that saltmarsh sparrows tended to have higher blood THg concentrations than seaside sparrows both when present at the same marsh sites and across their ranges (Table 1, Appendix 2). These results are congruent with previous studies documenting elevated saltmarsh sparrow blood THg concentrations relative to sympatric marsh sparrow species (Shriver et al. 2006; Ruskin et al. In prep.). However, these results differ

from those of Warner et al. (2010), who found no differences between saltmarsh and seaside sparrows in coastal Delaware, though their sample of saltmarsh sparrows was relatively small. We hypothesize that interspecific differences in THg are due to differences in MeHg exposure through diet or differential foraging preferences. The trophic level at which an organism feeds is one of the principal mechanisms of bioaccumulation and magnification (Evers et al. 2005). Some dietary specialization exists between our focal species (Greenlaw et al. 2020), potentially leading to differences in the amount of MeHg-rich prey items taken. For instance, Post and Greenlaw (2006) documented that amphipods (order Amphipoda) and wolf spiders (family Lycosidae) were the first and fourth most important prey item for saltmarsh sparrows, respectively, but ranked much lower for seaside sparrows. Amphipods are scavengers that consume organic matter that originates from aquatic environments (Moore and Francis 1985; Persson 1999) and have elevated levels of Hg relative to organisms occupying higher trophic levels (George and Batzer 2008). In addition, due to their predatory nature and elevated capacity for MeHg biomagnification, spiders pose a potential risk to arachnivorous songbirds (Cristol et al. 2008; Gann et al. 2015). Shriver et al. (2006) found a similar pattern between Nelson's and Saltmarsh sparrows breeding in coastal Maine salt marshes and attributed differences in Hg to variation in foraging strategies between the taxa. Quantifying MeHg concentrations within food items consumed by tidal marsh sparrows remains an unexplored, yet crucial step in characterizing MeHg exposure for these species, as well as Hg dynamics and biomagnification within saltmarsh estuarine food webs as a whole.

Defining relevant land cover characteristics at an appropriate spatial scale is challenging. Hg can be transported over large distances via atmospheric and aquatic systems, and coastal

wetlands can have differential Hg loading and site-specific biogeochemical processes — all of which can influence MeHg exposure of wildlife. Relevant spatial boundaries likely vary in both size and shape across marshes, depending on marsh characteristics. Despite exploring the effects of geographic scale on model fit, we found no evidence that the proportions of developed or forested land cover were associated with blood THg concentrations of tidal marsh sparrows. Similarly, we found no evidence that cumulative average annual precipitation was associated. These landscape-level factors were intended to serve as a proxy for the amount of regional atmospheric deposition and the potential abundance of Hg point sources, such as industrial facilities and landfills, surrounding sampling points. While such parameters were not directly accounted for in our model set, they may play an important role in influencing MeHg exposure of tidal marsh sparrows.

Several water quality parameters that correlate with increased organismal Hg bioaccumulation and MeHg production in wetlands were also unaccounted for in this study, including low pH and high dissolved organic carbon (DOC) levels (Wiener et al. 1990; Wren 1991; Scheuhammer and Graham 1999; Ravichandran 2004; Hall et al. 2008). High salinity sediments may promote Hg demethylation (Compeau and Bartha 1987) and the frequency of inundations of each marsh could influence methylation rates and MeHg mobilization from anaerobic wetland sediments (Snodgrass et al. 2000). Freshwater wetlands that are shallow and experience long bouts of periodic drying have higher THg and MeHg concentrations in water, sediment, and fish than those with consistently moist sediments (Caldwell and Canavan 1998; Snodgrass et al. 2000). While poorly studied in saline environments, brackish water marshes that experience longer periods between inundations (ie. a shorter hydroperiod) have more time to

desiccate, thus elevating MeHg export and bioavailability (Hall et al. 2008). Future studies seeking to characterize MeHg bioaccumulation in coastal estuaries should obtain a comprehensive understanding of the unique biogeochemical and hydrological dynamics of each marsh site whenever possible.

Previous research suggests that up to 62% of saltmarsh sparrows breeding from Maine to New York, as well as >75% of saltmarsh sparrows and >60% of seaside sparrows wintering in North Carolina, are potentially at risk of deleterious MeHg effects on reproduction (Winder 2012; Lane et al. 2020). A severe limitation in producing precise at-risk population estimates is the lack of toxicity reference values for our focal species. A common practice in ecotoxicological wildlife monitoring is to extrapolate toxicity reference values across species because many species have not been studied (Warner et al. 2010; Lane et al. 2011, 2020; Winder 2012). Researchers are often cautioned against this practice because the potential exists for extrapolation to be severely biased (USEPA 2007). Therefore, we provide comparative context with caution as we lack any direct evidence of how THg may influence sparrow reproduction. Based on a predictive linear model using blood THg concentrations from Carolina wrens, a non-migratory passerine breeding along two contaminated rivers in Virginia, USA, Jackson et al. (2011) proposed that female Carolina wrens with blood THg concentrations of 0.7–1.2  $\mu\text{g/g}$  ww and 1.2–1.7  $\mu\text{g/g}$  ww were likely at risk of a 10–20% and 20–30% reduction in nesting success, respectively (but see Fuchsman et al. 2017). Using the Jackson et al. (2011) potential risk categories, we estimated that approximately 22% of saltmarsh and 11% of seaside sparrows sampled from Maine to Virginia may be at risk of a 10–20% reduction in nesting success, while

3% of saltmarsh and 4% of seaside sparrows may be at risk of a 20–30% reduction in nesting success.

Although approximately 21% of the sampled birds had blood THg levels above the proposed 10% effect concentration (EC10), 58% of saltmarsh sparrows and 33% of seaside sparrows sampled in coastal New Jersey were above the EC10. Coastal New Jersey is estimated to support 37% of the global saltmarsh sparrow breeding population, and 22% of the seaside sparrows that breed in the northeastern USA (Wiest et al. 2016). Elevated MeHg exposure cases have also been documented previously in the Northeast (Lane et al. 2011, 2020; Kopec et al. 2018).

Sea-level rise is the principal driver of tidal marsh sparrow population declines (Shriver et al. 2007; Gjerdrum et al. 2008), and could cause the extinction of the saltmarsh sparrow by mid-century (Correll et al. 2017; Field et al. 2017, 2018; Roberts et al. 2019). Concurrently, some tidal marsh sparrows may face challenges coming from industrial practices in terrestrial systems that expose them and other wildlife to potentially dangerous levels of Hg contamination. For tidal marsh sparrows to persist into the next century, significant conservation efforts are required (Hartley and Weldon 2020). Ensuring that secondary factors such as heavy metal contamination do not compromise the persistence of remaining populations is beneficial to ensuring long-term success — as long as mitigating the effects of sea-level rise on tidal flooding remains the primary conservation priority.

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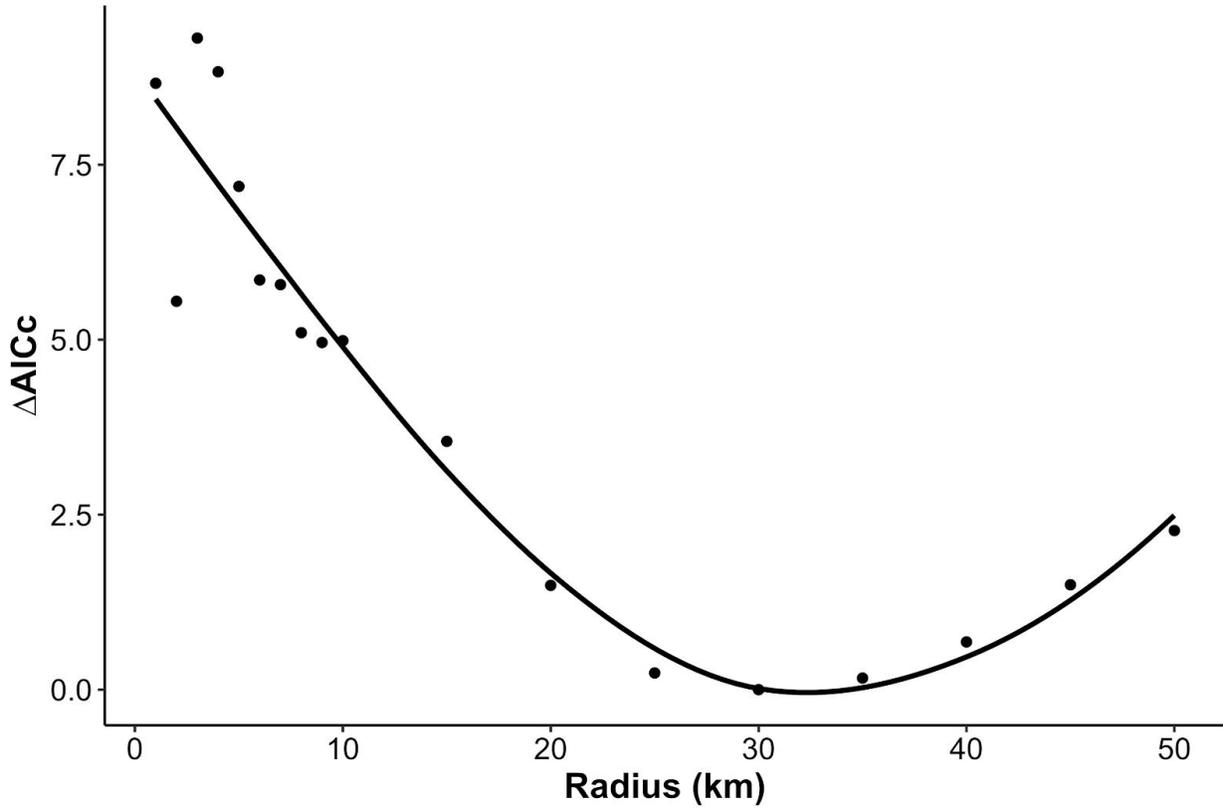
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**Appendix 1**  $\Delta AIC_C$  values for models with buffer radii from 1–50 km. Graph indicates that 30 km is the best scale of effect for which developed and forested land cover, as well as cumulative average annual precipitation, surrounding each focal marsh can best predict the natural log-transformed blood THg concentrations ( $\mu\text{g/g ww}$ ) of breeding tidal marsh sparrows in salt marshes from Maine to Virginia, USA, June 1 to July 31, 2018



**Appendix 2** Regional- and site-level tidal marsh sparrow blood THg concentration comparisons of other published works through time

Study Cited	Area	Marsh Site	Year Sampled	Saltmarsh Sparrow THg ( $\mu\text{g/g ww}$ )			Seaside Sparrow THg ( $\mu\text{g/g ww}$ )		
				n	Mean $\pm$ SD	Range	n	Mean $\pm$ SD	Range
This paper	ME – VA	—	2018	67	0.53 $\pm$ 0.29	0.13–1.4 2	53	0.44 $\pm$ 0.32	0.15–1.5
This paper	ME – NY	—	2018	35	0.54 $\pm$ 0.22	0.13–1.0 6	—	—	—
Lane et al. 2011	ME – NY	—	2004–08	653	—	0.14–3.7 0	—	—	—
This paper	ME	—	2018	17	0.45 $\pm$ 0.14	0.27–0.7 8	—	—	—

Lane et al. (2011)	ME	—	2004–08	239	—	0.22–1.4 4	—	—	—
Shriver et al. (2006)	ME	—	2001	53	0.69 ± 0.20	0.34–1.2 6	—	—	—
This paper	DE Bay (DE side)	—	2018	6	0.72 ± 0.36	0.44–1.4 2	6	0.45 ± 0.13	0.29–0.6 0
Warner et al. (2010)	DE Bay (DE side)	—	2006–07	35	—	0.19–0.8 3	100	—	0.15–2.1 2
This paper	ME	Scarborou gh Marsh WMA	2018	3	0.33 ± 0.07	0.27–0.4 0	—	—	—
Lane et al. (2011)	ME	Scarborou gh Marsh WMA	2007	14	0.52 ± 0.24	0.22–1.1 0	—	—	—
Lane et al. (2011)	ME	Scarborou gh Marsh WMA	2004	15	0.47 ± 0.16	0.23–0.8 2	—	—	—
Lane et al. (2020)	ME	Scarborou gh Marsh WMA	2000–09	62	0.562 ± 0.18	—	—	—	—
This paper	ME	Rachel Carson NWR	2018	8	0.45 ± 0.13	0.31–0.7 1	—	—	—
Lane et al. (2020)	ME	Rachel Carson NWR	2004–15	121	0.642 ± 0.16	—	—	—	—
This paper	ME	Rachel Carson NWR - Furbish Rd.	2018	4	0.45 ± 0.18	0.31–0.7 1	—	—	—
Lane et al. (2011)	ME	Rachel Carson NWR - Furbish Rd.	2008	57	0.70 ± 0.19	0.39–1.4 0	—	—	—
Lane et al. (2011)	ME	Rachel Carson NWR - Furbish Rd.	2007	45	0.71 ± 0.12	0.53–1.0 0	—	—	—
Lane et al. (2011)	ME	Rachel Carson NWR - Furbish Rd.	2006	16	0.73 ± 0.11	0.58–0.9 5	—	—	—
Lane et al. (2011)	ME	Rachel	2005	21	0.76 ±	0.47–1.4	—	—	—

		Carson NWR - Furbish Rd.			0.21	4			
Lane et al. (2011)	ME	Rachel Carson NWR - Furbish Rd.	2004	15	0.56 ± 0.09	0.33–0.69	—	—	—
This paper	NY	Marine Nature Study Area	2018	3	0.79 ± 0.18	0.60–0.96	—	—	—
Lane et al. (2011)	NY	Marine Nature Study Area	2008	5	0.68 ± 0.26	0.45–1.10	—	—	—
This paper	NY	North Cinder Island	2018	2	1.00 ± 0.08	0.95–1.06	—	—	—
Lane et al. (2020)	NY	North Cinder Island	2008–17	54	1.48 ± 0.31	—	—	—	—
Lane et al. (2011)	NY	North Cinder Island	2008	13	1.50 ± 0.32	0.93–1.90	—	—	—
This paper	DE Bay (DE side)	Prime Hook NWR - Broadkill River	2018	3	0.49 ± 0.07	0.44–0.57	3	0.34 ± 0.05	0.29–0.40
Warner et al. (2010)	DE Bay (DE side)	Prime Hook NWR - Broadkill River	2006–07	12	0.47 ± 0.02 SE	0.37–0.60	14	0.43 ± 0.03 SE	0.22–0.63

## Figures

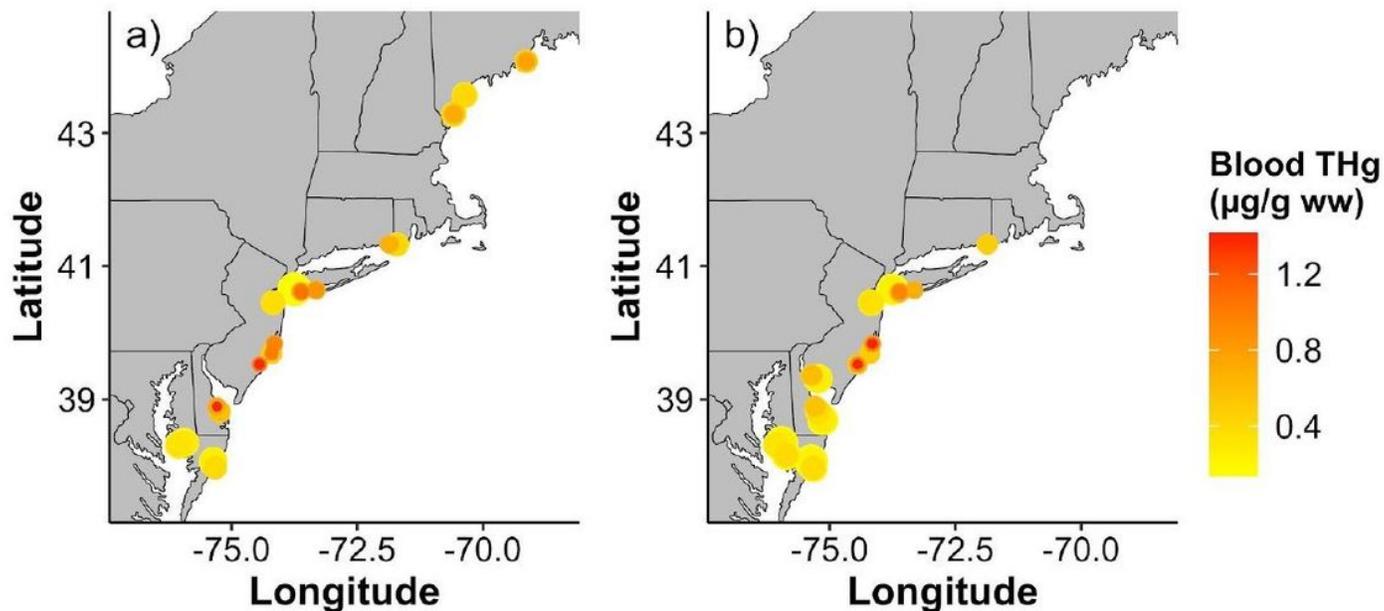
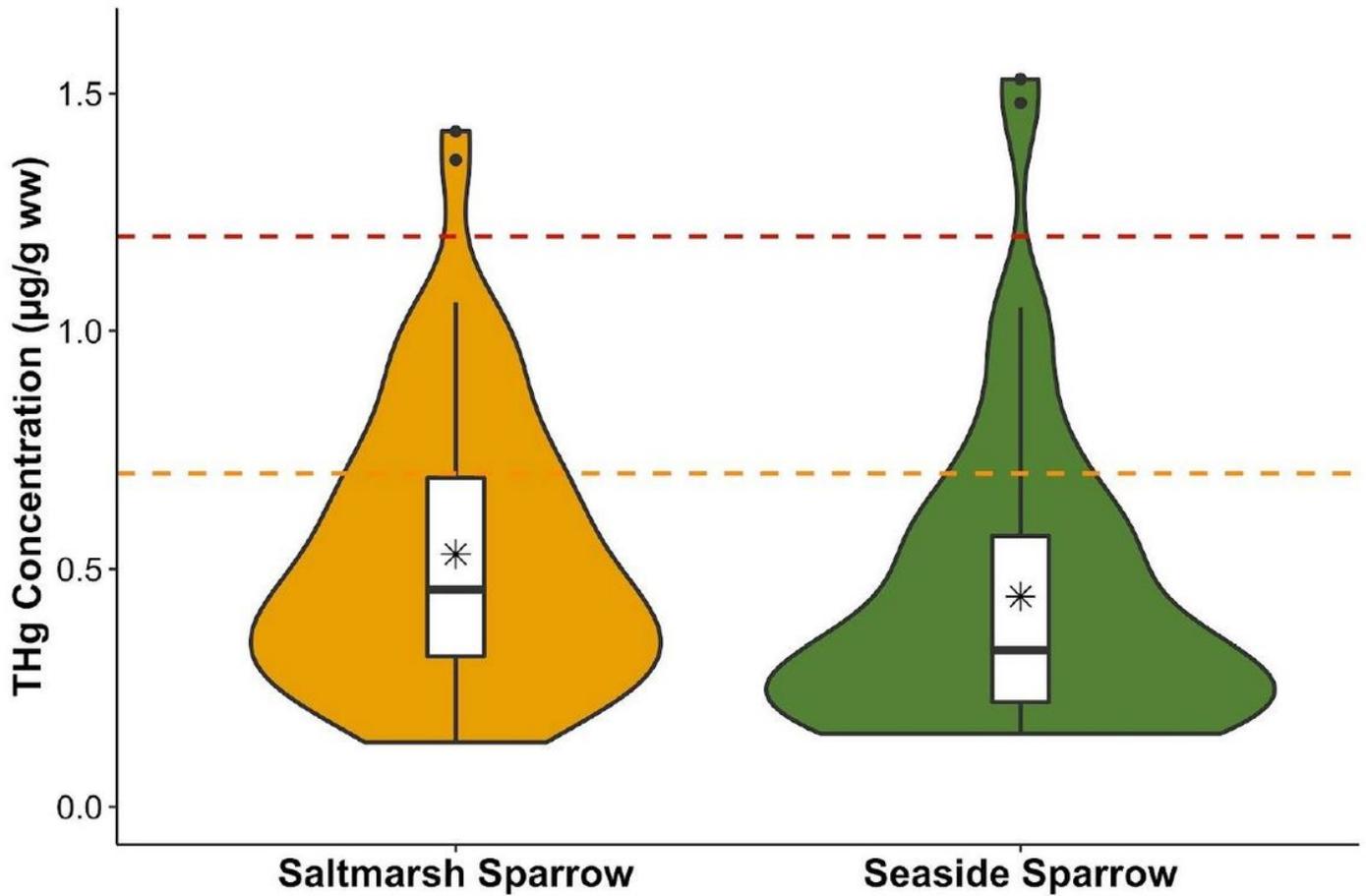


Figure 1

Map of blood THg concentrations (µg/g ww) of breeding a) saltmarsh sparrows and b) seaside sparrows for all sampling locations from Maine to Virginia, USA, June 1 to July 31, 2018. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.



**Figure 2**

Blood THg concentrations ( $\mu\text{g/g ww}$ ) of breeding tidal marsh sparrows from Maine to Virginia, USA, June 1 to July 31, 2018. The “violin” shapes represent the vertically-oriented distribution of THg concentrations per species. The boxplots display the outliers (dots), maximum and minimum concentration excluding the outliers (ends of whiskers), first and third quartile (central box), median (horizontal black line), and mean (\*) THg concentration per species. Dashed horizontal lines indicate the  $0.7 \mu\text{g/g ww}$  10% effect concentration (EC10) and the  $1.2 \mu\text{g/g ww}$  20% effect concentration (EC20) defined by Jackson et al. (2011)